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The effects of ultrasonic pretreatment and structural changes during the osmotic dehydration of the 'Starking' apple (*Malus domestica* Borkh)

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Abstract

During the osmotic dehydration (OD) of fruit, the cell membrane displays a high resistance to mass transfer, thereby reducing the dehydration rate. To reduce thermal damage to cell membranes, alternative methods have recently been introduced to reduce the initial moisture content and/or modify the structure of fruit tissue. The aim of this work was to evaluate the effects of an ultrasound (US) pretreatment for OD on the effective diffusion coefficients and to observe the changes in the molecular structure of 'Starking' apple cubes by Fourier-transform infrared spectroscopy (FTIR) during a 3 h process using a 45°Bx sucrose solution at 60°C. In the pretreatment step, apple samples were immersed in an ultrasonic bath at 45 kHz for 20 min. The effective diffusion coefficients for water (D_{ew}) and solids (D_{es}) were calculated from the observed osmotic kinetics according to Fick's second law for the transient state. The solids coefficients were higher than the water coefficients in both processes due to the concentration difference ($D_e = 7.7 \times 10^{-9}$ and 9.7×10^{-9} m^2 s^{-1} for OD_{US}). The structural changes were determined by FTIR by measuring the molecular vibration frequency for sucrose. The 1,500-900 cm^{-1} region of the infrared spectra was used to monitor the effect of sucrose concentration on fruit structure. We observed that the first bonds formed were C-H and C-O-C stretching (at 920 and 1,129 cm^{-1}, respectively) in the sucrose skeleton and glycoside bonds among sucrose molecules. The water concentration affected the diffusion coefficient significantly due to its dependence on the physical structure of the food.

Additional key words: cavitation; dehydration kinetics; effective diffusion coefficient; molecular structure; solid gain; water loss.

Resumen

Efectos del ultrasonido y cambios estructurales durante la deshidratación osmótica de manzana (*Malus domestica* Borkh) 'Starking'

Durante la deshidratación osmótica (DO), la membrana celular representa una alta resistencia a la transferencia de masa y reduce la velocidad de deshidratación. Recientemente, para reducir el daño térmico de las membranas, se han considerado métodos alternativos para reducir el contenido de humedad inicial o modificar la estructura del tejido de la fruta. El objetivo de este trabajo fue evaluar el efecto del ultrasonido (US), cuando se usa como pretratamiento a la DO, sobre los coeficientes de difusión efectiva y observar los cambios en la estructura molecular de manzana 'Starking', mediante espectroscopía infrarroja por transformadas de Fourier (FTIR), durante 3 h de proceso con una solución de sacarosa a 45°Bx y 60°C. Como pretratamiento las muestras fueron tratadas en un baño ultrasónico, a 45 kHz por 20 min. Los coeficientes de difusión efectiva para agua (D_{ew}) y sólidos ($D_{es} = 7{,}7 \times 10^{-9}$ y $9{,}77 \times 10^{-9}$ m^2 s^{-1} para DO_{US}) fueron calcu-

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Abbreviations used: °Bx (Brix degrees, % soluble solids); FTIR (Fourier-transform infrared spectroscopy); OD (osmotic dehydration); OD_{US} (osmotic dehydration with ultrasonic pretreatment); SG (solid gain, g/100 g); US (ultrasound); WL (water loss, g/100 g).

lados de la cinética osmótica; de acuerdo a la segunda ley de Fick en estado transiente, estos últimos fueron más altos que los coeficientes para agua en ambos procesos debido a la diferencia de concentraciones. Los cambios estructurales fueron determinados por FTIR, en la frecuencia de vibración molecular para sacarosa. Pudo observarse que los primeros enlaces formados son del tipo C-H y C-O-C en estiramiento (920 y 1.129 cm^{-1}) del esqueleto de la sacarosa y del enlace glucosídico entre moléculas de sacarosa. La concentración del agua afecta significativamente al coeficiente de difusión, debido a su dependencia con la estructura física del alimento. En este estudio se utilizó la región 1.500-900 cm^{-1} del espectro infrarrojo, para seguir el efecto de la concentración de masa de sacarosa en la estructura de la fruta.

Palabras clave adicionales: cavitación; cinética de deshidratación; coeficiente de difusión efectiva; estructura molecular; ganancia de sólidos; pérdida de agua.

Introduction

The apple (*Malus domestica* Borkh) is a fruit in the family *Rosaceae*, with diverse shape, size, colour and flavour characteristics according to the variety. The cv. 'Starking' is one of the best known varieties; originating in the United States, 'Starking' cultivar is a 'Red Delicious' mutation with bright red and green striped skin. Its flesh is yellowish white and crunchy; the taste is sweet. Is a product that stands out in the market because it can be found nearly year-round and ready for consumption (Sinha, 2006). Despite its good keeping qualities, as with all products, 'Starking' apples tend to decrease in quality over time. The possibility of developing minimal processing schemes for fruit that allow for the creation of products with sensorial characteristics similar to the source raw material and simultaneously possess a reasonable shelf life has broad prospects for current industrial applications in the manufacture of pre-processed raw materials for later use and to obtain new final products.

One possible method for apple processing is osmotic dehydration (OD) at mild temperatures with sugar solutions to preserve fruit properties. OD reduces the water content and enzymatic activity with minimal changes to the product characteristics (Martínez-Monzo *et al.*, 2001).

The OD process involves water extraction from a product that is immersed in a hypertonic solution and controlling contact time and temperature. This extraction is due to the driving force created by the high osmotic pressure, or low water activity (a_w), of the solution or the concentration gradient between the solution and the solid (Rastogi & Raghavarao, 1996). Because the a_w and solute gradients move across the food cell membrane, there at least are two concurrent flows in the osmotic process: a water flow towards the solution and a simultaneous solute transfer from the solution to the food. However, the mechanism controlling these simultaneous flows is not fully understood.

OD kinetics depend on various factors including the nature of the foodstuff, its size and geometry, the concentration and temperature of the hypertonic solution, and the solution/sample ratio (Lerici *et al.*, 1985). Unfortunately, the cell membrane has a high mass transfer resistance and reduces the dehydration rate (Erle & Shubert, 2001). Therefore, cell membrane modification may accelerate mass transfer. For this purpose, alternative methods such as pretreatments have recently been considered to reduce membrane thermal damage; such pretreatments can be used to reduce the initial moisture content or alter the food tissue structure before dehydration.

The development of non-thermal processing methods has attracted much attention in the food industry. Studies on OD and ultrasound-assisted OD have shown that different fruits display varying responses upon the application of these treatments before drying (Rodrigues & Fernandes, 2007a, b; Souza *et al.*, 2007; Fernandes *et al.*, 2008). Cavitation, a phenomenon produced by sonication, consists of the formation bubbles in a liquid that can collapse explosively and generate localised overpressure. This effect increases the diffusion and osmotic processes (Mason *et al.*, 1996).

Fourier-transform infrared spectroscopy (FTIR) is an analytic technique that has received much attention in the analytical chemistry field. This method utilises interferometry, providing many advantages over spectrometers of the dispersive type. FTIR provides a significant improvement in the signal/noise ratio, and the multiplexor feature allows for the simultaneous detection of all frequencies, reducing testing time without losing resolution (Griffiths & de Haseth, 1986; van de Voort, 1992). Vibrational spectroscopy has proven to be a powerful tool for studying the molecular structures and interactions of carbohydrates. Infrared absorption spectral analysis indicates what types of molecules are present in the sample. Because it offers the possibility to measure the vibra-

tion types in atomic bonds at different frequencies, functional groups and possible changes in sample structure and concentration can be recognised using infrared spectroscopy (Kačuráková & Mathlouthi, 1996). In the above context, the aim of this work was to evaluate the effect of US pretreatment on the effective diffusion coefficients in OD by observing the changes in the molecular structure of 'Starking' apple using FTIR.

Materials and methods

Apple fruits were acquired at a local market and classified according to ripeness state. The average characteristics of the selected apples were a firmness of 70 ± 3 N, a soluble solids percentage of 14.5 ± 0.2ºBx, an acidity of 0.23 ± 0.03 g of malic acid per 100 g, and a moisture content of 85.0 ± 1.2%. After the apples were washed and peeled, they were cut into 1 cm^3 cubes. Osmotic solutions were prepared with 45% sucrose (w/w) and held at 60°C for three hours. The samples were immersed into this solution and removed every 30 min and drained for 5 min before weighing. To determine their moisture contents, the samples were then placed in an oven at 70°C and dried to a constant weight, both initially and at each experimental time point. The soluble solids concentration was determined using a digital refractometer (Sper Scientific 300003, AZ, USA).

The samples subjected to pretreatment (OD_{US}) were loaded into Erlenmeyer flasks filled with distilled water (4:1) and placed in an ultrasonic bath T1-H5 (Elma GmbH & Co. KG, Singen, Germany) for 20 min at 45 kHz; after 5 min of US, the samples were blotted with absorbent paper, and each sample was weighed before dehydration.

From the sample moisture and weight data, we calculated the osmotic parameters and the percentages of water loss (*WL*) and solids gain (*SG*) (Kaymak-Ertekin & Sultanoglu, 2000):

$$WL = \frac{W_{w0} - \left(W_t - W_{st}\right)}{W_{so} + W_{w0}} x100 \qquad [1]$$

$$SG = \frac{W_{st} - W_{s0}}{\left(W_{s0} + W_{w0}\right)} x100 \qquad [2]$$

where *W* represents weight in *g*, *w* and *s* represent water and solid (*g*), respectively, and *t* and 0 are the experimental states at time *t* (*s*) and initially, respectively. Both parameters are expressed as g/100 g of fresh sample. The equilibrium values for these parameters (WL_∞ and SG_∞) were obtained using the kinetic model proposed by Azuara *et al.* (1992).

For the mass transfer approximation at equilibrium, we assumed first-order kinetics using the coefficients calculated with the following equations (Rastogi & Raghavarao, 2004):

$$-\frac{dm}{dt} = k_m(M_t - M_\infty) \qquad [3]$$

$$\frac{ds}{dt} = k_s(S_t - S_\infty) \qquad [4]$$

where k_m and k_s are the water and solid transfer coefficients (s^{-1}), respectively, and *M* and *S* refer to the *g* of water and solids in the sample, respectively. The subscripts *0*, ∞ and *t* represent the initial concentration and those at equilibrium and at any time, respectively.

The effective diffusion coefficients for water (D_{ew}) and solutes (D_{es}) were calculated using the sorption kinetic method, which is based on the assumption that adsorption and desorption rates follow a nonstationary-state diffusion equation through the solid. Considering the rectangular parallelepiped geometry, the equations that provide a solution to Fick's second law to obtain these coefficients are as follows (Crank, 1975; Rastogi *et al.*, 2002):

$$M_r = \sum_{n=1}^{\infty} C_n{}^3 \exp\left[-D_{ew} t q_n{}^2 \left(\frac{3}{a^2}\right)\right] \qquad [5]$$

$$M_s = \sum_{n=1}^{\infty} C_n{}^3 \exp\left[-D_{es} t q_n{}^2 \left(\frac{3}{a^2}\right)\right] \qquad [6]$$

where $C_n = 2\alpha(1 + \alpha)/(1 + \alpha + \alpha^2 q_n^2)$ and q_n are the positive, nonzero roots of the equation $tan\ q_n = -\alpha q_n$. Here, α is the ratio of solution volume to the volume of each apple cube.

At each experimental time point, dried samples were analysed as pellets in KBr on a Spectrum One FTIR Spectrometer (Perkin Elmer, Waltham, MA, USA), between 400-4,000 cm^{-1} at a resolution of 4 cm^{-1}. Reference spectra for sucrose, fresh apple and pectin were obtained using this technique to distinguish the functional group spectrum peaks. The analysis was performed in the normal frequency range for sucrose molecular vibrations using the onboard instrumental software. The diffusion kinetics were obtained during the osmotic dehydration process. To enhance the apparent resolution and amplify small differences in low-intensity bands overlapped by bands of higher inten-

sity, FTIR spectral deconvolution was performed using the Savitsky-Golay method (a second-order polynomial with 15 data points) using the Origin Pro 8 program, (Savitsky & Golay, 1964).

Each experimental run, consisting of the osmotic dehydration of 21 experimental units (cubes) over 3 h, was conducted in triplicate. The water fraction (*Xw*) for each experimental time point during OD was considered to be the response variable. Statistical analyses were performed using MINITAB 15. A two-way ANOVA analysis was applied within experimental runs (each time repetition) to verify the mean equality, which was confirmed with an a posteriori Tukey test. Another one-way ANOVA was applied among runs (replicates) to obtain statistically valid mean values for the entire experimental series. We then obtained and compared the 95% confidence intervals to confirm the average behaviour. Only the mean values were used to compute the osmotic parameters and diffusion coefficients.

Results

The time evolution of *WL* and *SG* are illustrated in Figure 1; we observed that both parameters were higher for the ultrasound-pretreated samples. Approximately 50% of the water was lost with ultrasonic pretreatment, with a simultaneous solids gain of 22%, versus 47% and 17%, respectively, for osmotic dehydration without pretreatment. The equilibrium values (WL_∞ and SG_∞) are listed in Table 1. These values are higher for OD with ultrasonic pretreatment, and approximately 16.5% less time is required to reach equilibrium.

Because dehydration was performed for only 3 h, the rates of change in the moisture (*-dm/dt*) and solids (*ds/dt*) contents were obtained as functions of the average moisture and solids contents, respectively, to infer their equilibrium values (M_∞, S_∞) and to obtain the mass transfer coefficients for water (k_m) and solids (k_s). It is evident that the coefficients for both water and solids increased with the use of ultrasonic pretreatment. Table 1 also lists the effective diffusion coefficients for the solutes and water in the two processes computed from the analytical solution of Fick´s second law. Higher values were obtained for the ultrasonically pretreated product.

To distinguish the molecular characteristic vibrations of apple components, we measured the spectra of the pure substances and fresh apple (Figure 2). The main apple frequencies (1,745 and 1,640 cm^{-1}) correspond

Table 1. Mass transfer parameters

Parameters	Process	
	OD_{US}[1]	OD
WL_∞ (%)	53.33 ± 2.66	50.55 ± 2.02
$D_{ew} \times 10^{-9}$ ($m^2\ s^{-1}$)	5.026 ± 0.251	4.358 ± 0.174
M_∞ (g)	0.438 ± 0.022	0.455 ± 0.020
$k_m \times 10^{-4}$ (s^{-1})	5.030 ± 0.251	4.358 ± 0.219
SG_∞ (%)	22.68 ± 1.13	17.67 ± 0.71
$D_{es} \times 10^{-9}$ ($m^2\ s^{-1}$)	9.717 ± 0.485	7.698 ± 0.308
S_∞ (g)	0.358 ± 0.018	0.362 ± 0.014
$k_s \times 10^{-4}$ (s^{-1})	8.982 ± 0.450	7.383 ± 0.295

[1] Osmotic dehydration with ultrasound pretreatment.

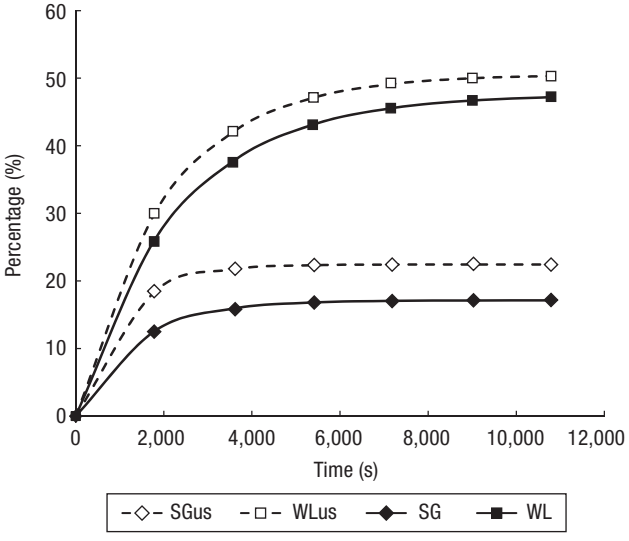

Figure 1. Osmotic parameters (SG: solid gain, g/100 g; WL: water loss, g/100 g) with and without ultrasound pretreatment.

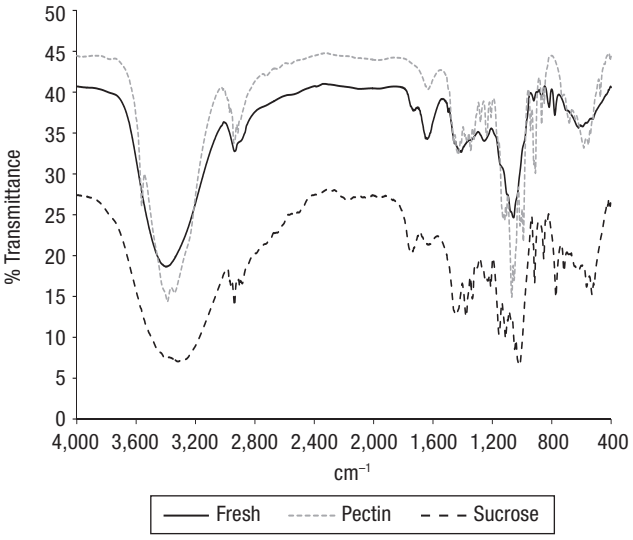

Figure 2. Spectra reference for fresh apple.

to pectin carboxyl groups. Furthermore, the fructose characteristic peaks (3,565, 1,636, 1,430, 1,130 and 920 cm^{-1}) and those of the glycosidic bonds and the glucose ring (1,460, 1,104, 1,053, 990 cm^{-1}) are clearly observable in the sucrose spectrum.

The spectra obtained from the samples at various dehydration times were analysed with respect to the molecular vibrational frequencies of sucrose. For clarity, only the FTIR spectra for the initial condition (fresh) and those at 30, 60 and 180 min of OD are shown in Figure 3. The development of peaks for the hypertonic solute during dehydration can be observed here. For the ultrasound-treated samples, this development starts in the first 30 min, whereas for the simple dehydration process, development begins at 60 min.

The kinetics obtained from the infrared spectra are presented in Figure 4, which depicts the time dependence of bond formation between the apple components and sucrose according to the characteristic molecular vibration wave number (cm^{-1}).

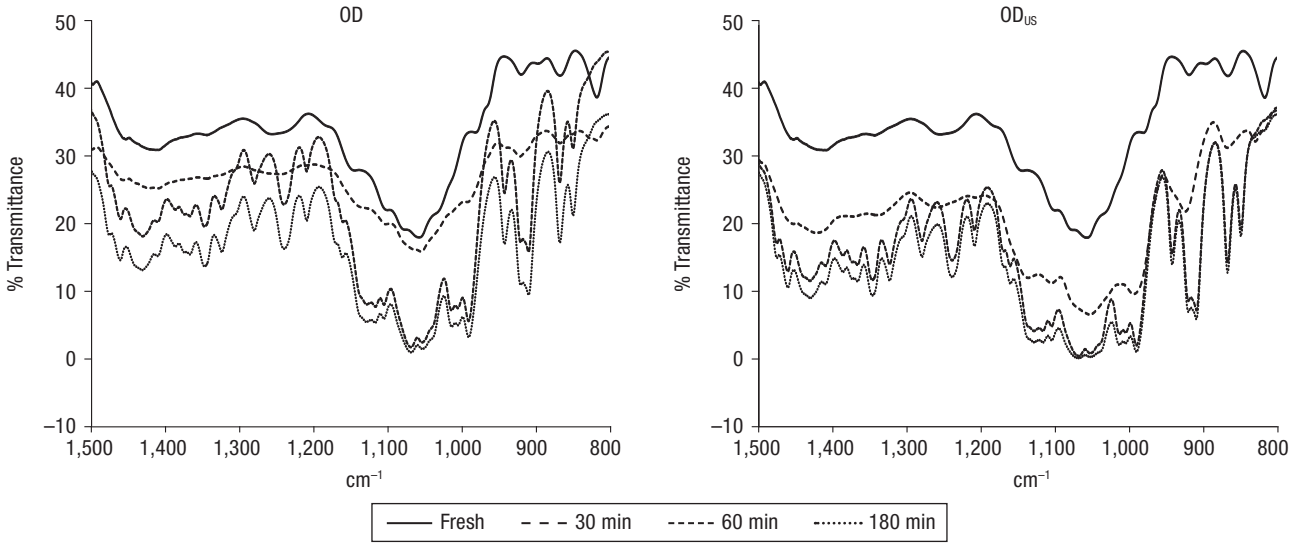

Figure 3. Apple FTIR spectrums at different osmotic dehydration (OD) times, with (right) and without (left) ultrasound pretreatment.

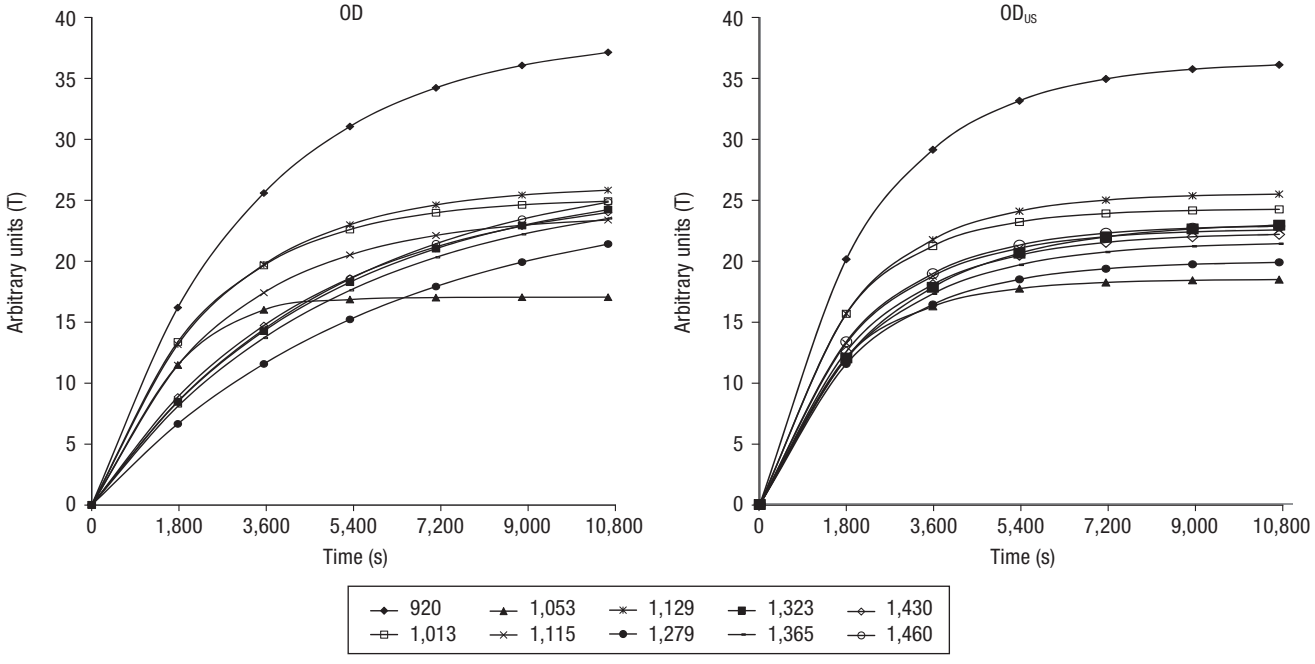

Figure 4. Sucrose kinetic, during osmotic dehydration, with (right) and without (left) ultrasound pretreatment.

Discussion

During dehydration, the osmotic driving potentials for moisture and solute transfer decrease due to structural changes caused by the rapid water loss and solids uptake near the surface, thus increasing the mass transfer resistance (Singh *et al.*, 2007). In the ultrasonic pretreatment process, the tissue structure is opened in the intercellular spaces, and solute diffusion and external solution hydrodynamics are increased by the action of ultrasound waves, contributing to net opposite fluxes of water and solutes that allow the tissue to become more concentrated. According to Chiralt & Fito (2003), mass transfer behaviour in plant tissues during osmotic treatment is affected by the volume fraction of intercellular space (associated with the degree of compactness of cell packaging), by the presence of a gas or a liquid phase in these pores and by variations in cell membrane permeability.

When sonic energy passes through a material, a continuous motion wave occurs, resulting in an alternate particle compression and expansion movements in the medium (the "sponge" effect). This effect is responsible for the development of microscopic channels, reducing the layer boundary diffusion and increasing the convective mass transfer in food (Povey & Mason, 1998; Tarleton & Wakeman, 1998; Fuente-Blanco *et al.*, 2006). It is known that the mass transfer of water and solutes rates in intercellular spaces or trans-membrane tissues depends on diffusional and/or osmotic mechanisms. Here, the solid diffusion rate is higher in both processes, where the driving force behind mass transfer is the concentration difference.

In the FTIR transmittance measurement, the crystalline sucrose spectrum (Figure 2) is characterised by relatively sharp peaks throughout the 4,000 to 900 cm^{-1} region, whereas many broader peaks are visible in the fresh apple. The broad OH peak indicates a wide range of hydrogen bond lengths and orientations. The main reason for the sharper and more intense peaks in the crystalline state is the higher degree of homogeneity in intermolecular interactions (Wolkers *et al.*, 2004). Asymmetric and symmetric stretching vibrations of ionic carboxylic groups (-COO-) appeared at 1,644 and 1,420 cm^{-1}, respectively. The peaks at 1,340 cm^{-1} may be assigned to pectin -COO- symmetric stretching, and those at 1,053 cm^{-1} are assumed to be the C-OH stretching vibrations of alcoholic groups and carboxylic acids (Iqbal *et al.*, 2009).

For the full osmotic dehydration (Figure 3), the largest changes occur in the spectra at 1,420 and 780 cm^{-1}, which are the areas corresponding to the molecular vibrations of sugars and pectin bonds or functional groups present in the samples. The peaks between 1,200 and 900 cm^{-1} arise from a combination of C-O and C-C stretching and C-O-H bending. These peak positions progressively shift to a lower wave number, and their bandwidth increases. The shifts of dehydration-induced frequency arise from changes in the hydrogen bonding positions of the C-O-H groups (Kačuráková & Mathlouthi, 1996). The hydrogen-bonding interactions between sucrose and water in solution are replaced by intermolecular sucrose hydrogen bonds upon dehydration. Therefore, the mobilities of both water and solutes are affected, and thus, their effective diffusion coefficients.

With respect to the sucrose kinetics (Figure 4), the first bonds formed are C-H and C-O-C stretching types (920 and 1,129 cm^{-1}) from the bonds in the sucrose skeleton and glycosidic bonds among sucrose molecules; C-O stretching from β-type bonds in sucrose (1,013 cm^{-1}) and O-C-H bending from pectin molecules (1,323 cm^{-1}) were also observed. The difference between the processes over time is evident as these bonds are formed, which is related to the higher sucrose diffusion rate due to the presence of microchannels formed by the application of ultrasound. The use of ultrasonic pretreatment affects the permeability of the cell membranes; thus, the rates of water and solids transfer during OD increase with increasing microchannel development.

FTIR has become an important technique for the analysis of food performance and an extraordinarily useful tool for tracking and describing the changes in a product during its transformation (Smith, 1996). In this study, the 1,500 to 900 cm^{-1} region of the FTIR spectrum was used to observe the effect of sucrose mass concentration on fruit structure. This region is one of the richest in structural information, as it arises from the symmetric deformations of CH_2 groups, C-O stretching peaks and OH bending (C-O-H) vibrations.

In conclusion, the effects of ultrasonic pre-treatment are evident at structural changes over time in the bond vibrations of apple pectin rather than those of sucrose bonds. This work represents a significant application of infrared spectroscopy in food science, not only as an analytic technique but also as a useful approximation tool for the study of diffusional phenomena that occur during processing. The kinetics obtained in this way

can be related to results derived using other analytical methods. Clearly, there is a strong relationship between the moisture and solid contents and the physical properties of a food. Further innovative research is thus required to describe and fully characterise the OD process. In this case, the structural changes (mainly shrinkage and porosity) caused by ultrasound and process conditions should be further elucidated.

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